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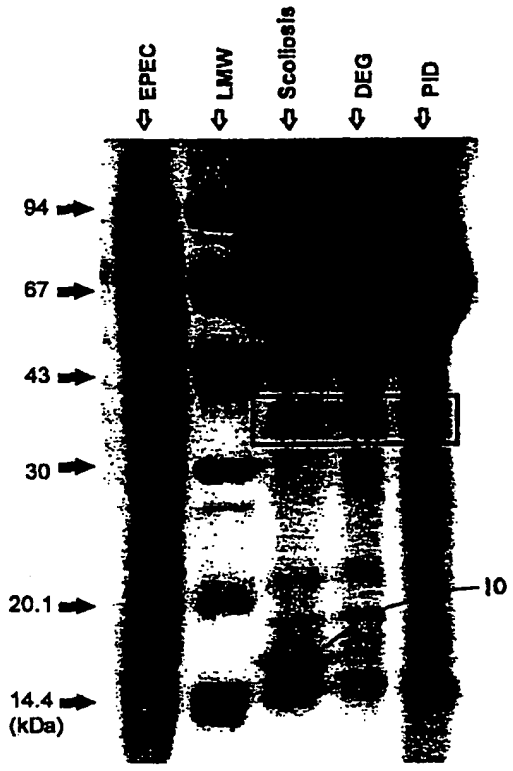
(54) Title: BACK PAIN DIAGNOSIS AND APPARATUS FOR USE THEREIN

(57) Abstract

The intervertebral disc is implicated as a source of symptoms of low back pain and sciatica. Degenerative disc disease and inflammatory processes may result in low back pain. The invention identifies the involvement of lysozyme C and tyrosine phosphorylation in low back pain, sciatica and other joint diseases and provides methods and apparatus for diagnosis and uses of these substances, and processes and substances influencing these processes in the treatment and diagnosis of such diseases and conditions.



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BACK PAIN DIAGNOSIS AND APPARATUS FOR USE THEREIN

The present invention relates to the diagnosis and therapy of back pain, and apparatus for use therein.

Back pain is, after the everyday cold, the most common health problem in Britain and America. Eighty percent of the population will experience the symptoms of back pain. It is believed that the annual cost of treating back pain in the UK is approximately £5 billion.

The intervertebral disc is implicated as a source of symptoms in the majority of patients with low back pain and sciatica, particularly in relation to sciatica secondary to disc prolapse. Degenerative disc disease is characterised by the gradual loss of water and proteoglycan from the nucleus pulposus which in part leads to biochemical changes within the disc resulting in height loss and disc prolapse.

It has been implied that inflammatory processes may be activated resulting in low back pain. In addition there may also be a series of related biochemical processes that may be activated resulting in disc degeneration and the generation of inflammatory pain.

In this specification the term "lysozyme C" is to be understood as including any lysozyme C like proteins which have a similar N-terminal amino acid sequence as found in lysozyme C.

According to the present invention there is provided a method of diagnosing low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint disease, or a predisposition thereto, said method comprising detecting the presence of lysozyme C in tissue extracted from intervertebral discs or fluid extracted from large joints.

Preferably the amount of lysozyme C is detected.

The method preferably comprises western blotting, dot blotting, or an enzyme linked immunosorbent assay. Preferably monoclonal antibodies to lysozyme C are used in these tests. Two antibodies may be used, each of which recognises a different epitope of lysozyme C. Preferably both antibodies recognise denatured and native protein. One of said antibodies may be conjugated to an enzyme such as alkaline phosphatase with the other antibody remaining unconjugated. Preferably a colour change occurs when lysozyme C is present. Preferably a substrate for the enzyme provides the colour change in the presence of lysozyme C.

Preferably only the conjugated monoclonal antibody is used in western blotting and dot blotting. The conjugated antibody and the unconjugated antibody are preferably utilised in the enzyme linked immunosorbent assay.

Alternatively, the method may comprise a colorimetric assay. Preferably colorimetric substrates for lysozyme C are synthesised for use in the assay. Preferably the substrate is an indol based compound linked to N-acetylglucosamine by a β 1-4 linkage. This substrate is preferably clear and the mixture of substrate and lysozyme C may become coloured when lysozyme C cleaves the linkage. The substrate may alternatively be a compound where N-acetylmuramic acid is linked to an indol based compound. The absorbance of the released indol based compound is preferably measured at its peak absorbance when excited at its excitation wavelength.

Further according to the present invention there is provided a method of diagnosing low back pain and sciatica associated with degenerative disc disease and degenerative joint disease, or a predisposition thereto, said method comprising introducing a lysozyme C substrate analogue in tissue of intervertebral discs or large joints and analysing the magnetic resonance of the substrate with nuclear magnetic resonance techniques.

The analogue may be introduced by injection, and may comprise a substrate homologue with a beta 1-4 linkage.

Preferably the method involves analysis of the degree of change in the magnetic resonance properties of the homologue, which may provide for determination of the degree of disease progression.

Further according to the present invention there is provided apparatus for use in the diagnosis of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said apparatus comprising a support on which an unconjugated monoclonal antibody to lysozyme C is immobilised whereby an indication is given when the support comes into contact with lysozyme C.

The support preferably comprises a test strip or a nitro-cellulose/nylon sandwich membrane. The test strip may comprise opaque polystyrene or another support matrix which is able to bind antibodies. The support is preferably pre-blocked with blocking agents when the unconjugated antibody has been immobilised thereon.

The apparatus is preferably utilised in an enzyme linked immunosorbent assay using an enzyme conjugated monoclonal antibody to lysozyme C. Preferably the enzyme is alkaline phosphatase, horse radish peroxidase or biotin. The substrate for the enzyme preferably provides a colour change as an indication of the presence of lysozyme C.

The invention may further comprise a method of treatment of low back pain and sciatica, comprising the administration, to a body in need of such treatment, of an effective dose of lysozyme C.

The lysozyme C may be administered by injection into a vertebral disc or joint. The lysozyme C may be administered in combination with a growth factor and/or a phosphatase inhibitor. The method may be used to treat joint disease.

Still further according to the present invention there is provided a

method of diagnosing l w back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said method comprising detecting the presence of tyrosine phosphorylation in tissue extracted from intervertebral discs or fluid extracted from large joints.

Preferably the amount of tyrosine phosphorylation is detected. Preferably the method involves detecting the presence of annexin tyrosine phosphorylation.

The method preferably comprises western blotting, dot blotting, or an enzyme linked immunosorbent assay. Preferably two monoclonal antibodies are used in the tests. The first antibody preferably recognises tyrosine phosphate and is conjugated to an enzyme. The second antibody preferably recognises annexins and is unconjugated. The first antibody is preferably conjugated to alkaline phosphatase, horse radish peroxidase or biotin. Preferably a colour change occurs when a tyrosine phosphorylated annexin is present. Preferably a substrate for the enzyme provides the colour change in the presence of a tyrosine phosphorylated annexin.

Preferably a phosphatase inhibitor is utilised when extracting the tissue. Preferably the inhibitor is sodium vanadate.

Preferably only the conjugated monoclonal antibody is used in western blotting and dot blotting. The conjugated antibody and the unconjugated antibody are preferably utilised in the enzyme linked immunosorbent assay.

Yet further according to the present invention there is provided apparatus for use in the diagnosis of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said apparatus comprises a support on which an unconjugated monoclonal antibody to annexin is immobilised whereby an indication is given when the support comes into contact with tyrosine

ph sphorylated annexin.

The support preferably comprises a test strip or a nitro-cellulose/nylon sandwich membrane. The test strip may comprise opaque polystyrene or another support matrix which is able to bind antibodies. The support is preferably pre-blocked with blocking agents when the unconjugated antibody has been immobilised thereon.

The apparatus is preferably utilised in an enzyme linked immunosorbent assay using an enzyme conjugated monoclonal antibody to tyrosine phosphate. Preferably the enzyme is alkaline phosphatase, horse radish peroxidase or biotin. The substrate for the enzyme preferably provides a colour change in the presence of tyrosine phosphorylated annexin.

The invention further provides apparatus for use in the diagnosis of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said apparatus comprising a support on which an unconjugated monoclonal antibody to annexin and an unconjugated antibody to lysozyme C are immobilised whereby an indication is given when the support comes into contact with tyrosine phosphorylated annexin and lysozyme C.

The two antibodies are preferably immobilised on separate areas of the support. The support preferably comprises a test strip or a nitro-cellulose/ nylon sandwich membrane. The test strip may comprise opaque polystyrene or another support matrix which is able to bind antibodies. The support is preferably pre-blocked with blocking agents when the unconjugated antibodies have been immobilised thereon.

The apparatus is preferably utilised in an enzyme linked immunosorbent assay using enzyme conjugated monoclonal antibodies to lysozyme C and tyrosine ph sphate. Preferably the enzyme is alkaline phosphatase, h rse radish peroxidase or biotin. The substrate for the enzyme preferably provides a

colour change in the presence of tyrosine phosphorylated annexin and lysozyme C.

Further according to the present invention there is provided a method of diagnosing low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said method comprising detecting the presence of phosphatase in tissue extracted from intervertebral discs or fluid extracted from large joints.

Preferably the amount of phosphatase is detected.

The method preferably comprises placing aliquots of extract on a blank area of a test strip or a nitro-cellulose/nylon sandwich membrane. The test strip may comprise opaque polystyrene or another support matrix which is able to bind antibodies. The extract may be left on the test strip or membrane for a predetermined period of time and preferably for 10-20 minutes. Preferably a colour change occurs when there is immobilised phosphatase on the test strip or membrane. Preferably a substrate for alkaline phosphatase provides the colour change in the presence of immobilised phosphatase.

Alternatively, the method may comprise a colorimetric assay. Preferably the assay comprises placing aliquots of extract into a phosphatase substrate solution and measuring the light absorbance at a certain wavelength. The substrate is preferably a substrate for alkaline phosphatase which provides a colour change in the presence of phosphatase.

The invention further provides for a method of treatment of low back pain and sciatica, comprising the administration, to a body in need of such treatment, of an effective dose of phosphatase inhibitor.

The phosphatase inhibitor may be administered by injection into a vertebral disc r joint. The inhibitor may be administered in combination with lysozyme C. The method may also be used to treat joint disease.

The invention also provides for a method of treatment of l w back paid and sciatica, comprising the administration, to a body in need of such treatment, of an effective dose of tyrosine phosphorylation annexin.

The method may be used to treat joint disease.

The invention may further provide a method of diagnosing low back paid and sciatica, or a predisposition thereto, the method comprising analysing the presence of tyrosine phosphorylation or phosphatase and of lysozyme C in tissue extracted from the invertebral discs or fluid extracted from large joints. The method may also be used to diagnose joint disease.

The invention further provides for the use of lysozyme C and a phosphatase inhibitor in the manufacture of a medicament for the treatment of low back pain and sciatica. The invention may further be used to treat joint disease.

Further according to the present invention there is provided a method of diagnosing low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said method comprising detecting the presence of mutations in the lysozyme C gene in blood samples taken from the body.

The method comprises using a set primer for these mutations in conjunction with a standard primer with no mutation to amplify the gene fragments by the Polymerase Chain Reaction (PCR) if the mutation is present.

Alternatively the method may comprise synthesising primers with the non-mutant sequences in the 5' end for all mutation regions of the lysozyme C gene. Preferably the set of primers is used as a mixture thereby enabling DNA fragments to be amplified using PCR on the blood DNA to produce a DNA profile. Preferably the absence of certain bands from the profile is indicative of disease.

The invention further provides apparatus for use in the diagnosis of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said apparatus comprising a set of primers with non mutant sequences in the 5' end for all mutation regions of the lysozyme C gene for use in PCR amplification of DNA fragments.

The apparatus is preferably utilised for detecting the presence of mutations in the lysozyme C gene.

The invention also provides use of lysozyme C as a therapeutic agent in the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases.

The lysozyme C is preferably injected into the disc or joint. Preferably the lysozyme C is injected in combination with a growth factor.

The invention further provides for the use of lysozyme C substrate analogue in the analysis of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases.

Preferably nuclear magnetic resonance techniques are used to analyse the resonance of analogue introduced to tissue under analysis.

The invention still further provides for the use of lysozyme C in the manufacture of medicament for the treatment of back pain and sciatica.

The invention also provides use of a phosphatase inhibitor as a therapeutic agent in the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases.

The invention further provides for the use of a phosphatase inhibitor in the manufacture of a medicament for the treatment of back pain and sciatica.

The invention still further provides use of corticosteroids as a therapeutic agent in the treatment f l w back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases.

Preferably the corticosteroid is injected in combination with intradiscal or intrajoint injection of lysozyme C or with lysozyme C and phosphatase inhibitor.

The invention may further provide for the use of corticosteroids for use in the manufacture of a medicament for the treatment of back pain and sciatica.

The invention yet further provides use of growth factors as therapeutic agents in the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases.

The growth factors are preferably epidermal growth factor, platelet derived growth factor or hormones such as insulin, or any agonist which will restore the tyrosine phosphorylation of annexins.

The invention further provides the use of tyrosine phosphorylation annexins in the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint disease.

Preferably the growth factors may be injected in combination with any or all of lysozyme C, phosphatase inhibitor and corticosteroids.

The invention further provides for the use of tyrosine phosphoylated annexins in the manufacture of a medicament for the treatment of low back pain and sciatica.

The invention further provides for the use of growth factors in the manufacture of a medicament for the treatment of back pain and sciatica.

The invention still further provides for the use of any combination of lysozyme C, ph sphatase inhibitor, cortic steroids, growth factors and tyrosine phosphoylated annexins in the manufacture of a medicament for the treatment of low back pain, sciatica and joint disease.

Embodiments of the present invention will now be described by way of example only and with reference to the accompanying drawings, in which:

Fig. 1 is a coomassie stained polyacrylamide gel of cell lysates from the Nucleus Pulposus from a scoliosis, degenerate disc (DEG) and prolapsed disc (PID) patient.

Fig. 2 illustrates western hybridisation using monoclonal antibodies to tyrosine phosphate against cell lysates from the Nucleus Pulposus from a scoliosis, DEG and PID patient.

The present invention provides substances, methods and apparatus for the treatment, diagnosis and use in the treatment and diagnosis of back pain and sciatica, and in particular back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint disease, or a predisposition thereto.

The differences in protein profiles and basic cellular phosphorylation within the intervertebral disc (the nucleus pulposus), in particular the presence of tyrosine phosphorylated (Tyr-P) residues on proteins were investigated. Tyr-P proteins are involved in signal transduction, i.e. 'cell to cell communication', and in the regulation of cell proliferation. Hence they regulate factors that control growth, the cell cycle, cell to cell adhesion, as well as inflammation, Ca++, and secondary messengers. The phosphorylation of tyrosine residues on proteins is thus an important event within the cell.

Because f some genetic and/or environmental reason, signal transduction may be, 'switched on' or 'switched off'. The resulting abnormal

'signal transducti n' processes causes a shift in the equilibrium of enzymes, themselves proteins, and this is translated into alterations in the protein concentrations, and the result of this is seen as differences in the protein profiles within the cell.

The samples assayed were the nucleus pulposus (NP) which were carefully excised from the intervertebral disc. Patient groups studied were split into four groups:

'DISEASED PATIENTS':

1) PID (prolapsed intervertebral disc) = sciatica cases undergoing disc resection
2) DEG (degenerate disc) = mainly back pain cases undergoing anterior spinal fusion

'NORMAL PATIENTS':

3) Scoliosis (normal) = scoliosis cases undergoing disc resection and correction
4) Donor (normal) = disc removed from patients undergoing organ donation, with no past history of back pain.

RESULTS:

a) Tyrosine-Phosphorylated protein(s):

NP samples were processed and probed with anti-phosphotyrosine antibodies for the presence of Tyr-P proteins (12,14) using the Western hybridisation method. The four patient groups, detailed as above, were investigated. Results showed a significantly higher level of ph sphorylation of tyrosine residues from NP proteins in the normal gr ups (group 3 + 4) as compared to the diseased groups (group 1 + 2).

See Table 1 below and Fig. 1 and 2.

Table 1. Comparison of the levels of Tyr-P proteins in the four study groups.

Levels of Tyr-P

Group (N=)	High	Medium	Low	Absent
1-PID (17)[+]	0	4	7	6
2-DEG (16)[+]	3	5	5	3
3-Scoliosis (16)[+]	14	2	0	0
4-Donor (4)[++]	3	0	0	0

[+] = highly significant ($P<0.001$)

[++] = number too small for statistical analysis

Tyr-P proteins are involved in signal transduction, i.e. 'cell to cell communication', and in the regulation of cell proliferation. The above finding implies that there are significantly lower levels of Tyr-P of proteins, hence abnormal signal transduction, in the diseased states. Further Western hybridisation studies have confirmed this Tyr-P protein to be Annexin I and II. This pivotal protein is known to be involved in cell apoptosis, i.e. programmed cell death, as well as in the tight regulation of inflammation, in particular the regulation of PLA2 activity.

As the normal cellular constituents become deranged, because of the loss of growth and cell to cell adhesion, a state of degeneration occurs, i.e. what we term 'degenerative disc disease'. The condition becomes a painful process because there is, in addition, the loss of control of inflammation resulting in the generation of a cascade of noxious chemicals that stimulate pain. Symptomatic back pain and/or sciatica is the end result.

The invention therefore provides for the use of tyrosine phosphoylated

annexin for use in the manufacture of a medicament for the treatment of low back pain and sciatica and the use of ph sphatase inhibitors in the treatment, diagnosis and manufacture of medicaments for such treatment.

b) Lysozyme C

NP samples from the four groups were prepared and denatured proteins separated by SDS-agarose gel electrophoresis. The proteins were then visualised with Coomassie Blue staining. The major protein difference was a protein 10 with a molecular weight of approximately 15 kDa identified by N-terminal amino acid sequencing as lysozyme C as shown in Fig. 1. Results showed a significantly higher level of lysozyme C within the NP of the normal groups (group 3 + 4) as compared to the diseased groups (group 1 + 2). See Table 2 below.

Table 2. Comparison of the levels of lysozyme C in the four study groups.

Levels of Lysozyme C

Group (N=)	High	Medium	Low	Absent
1-PID (17)+	0	0	2	15
2-DEG (16)+	0	2	7	7
3-Scoliosis (16)+	13	3	0	0
4-Donor (4)++	3	0	0	0

+ = highly significant ($P<0.001$)

++ = number too small for statistical analysis

Lysozyme C is a secretory protein normally found in tears, saliva, breast milk, etc. and functions specifically in digestion of bacterial cell wall proteins. The significance of its presence within the intervertebral disc is not fully understood. The question raised is whether this enzyme has secondary

functions in the maintenance of the overall cellular integrity; akin to other mammalian proteins.

In contrast to this, a rare genetic variant of lysozyme, involving a point mutation of the lysozyme gene, has been reported to cause hereditry non-neuropathic systemic amyloidosis. Patients with back pain may have similar mutation in the lysozyme C gene.

The invention therefore provides for the use of lysozyme C in the treatment, diagnosis and manufacture of medicaments for the treatment of low back pain and sciatica.

Other Clinical Implications

Osteoarthritis

Osteoarthritis of the large joints, such as the hip and knee, results in pain and disability and imparts a major financial burden to the Health Services in Europe and the United States. The disease essentially results in the loss of articular cartliage of the affected joint. Interesting enough, cells within the intervertebral disc are very similar in nature to that in large joints and it is quite likely that a similar process of loss of tyrosine phosphorylation of annexin I and II and loss of Lysozyme C is occurring.

Unlike back pain, osteoarthritis of large joints, at least when significantly developed, is readily detectable on X-rays such that a diagnostic kit is not absolutely necessary at this stage. However, diagnostic kits for detecting tyrosine phosphorylation of annexin and also the presence of lysozyme C would provide an earlier indication of osteoarthritis. More importantly, in treatment terms, the degenerative process may be stopped either by 1) pharmacological agents known to inhibit the dephosphorylation process of annexin I and II and/or 2) Lys zyme C replacement therapy. This will obviate the need for an artificial joint with resulting tremendous savings for the Health Service.

In the light of the above results, the following diagnostic methods and test kits, and therapies were produced.

(1) LYSOZYME C DETECTION

Reagents:

a) 2 monoclonal antibodies to spinal disc lysozyme C, both recognise denatured and native protein. But both recognise different epitopes of lysozyme C. One remains unconjugated (UC) and is used in the test strip only, the other is conjugated to alkaline phosphatase (AP) and is used for detection in all tests.

b) Extraction buffer: 10 mM Tris-Hcl pH 7.0
1 mM EDTA
1-3% non ionic detergent (e.g. TritonX100) for native
OR if denatured
Sodium dodecyl sulphate (SDS).

c) blocking buffer: 3% serum albumin
0.5% Tween
in 10 mM Tris pH 7.0

d) fixing solution: 1% trichloro-acetic acid (TCA).

e) incubation buffer: 10 mM Tris-Hcl pH 7.0

f) AP substrate: BCIP (5-bromo 4-chloro 3-indoylphosphate) and
NBT (nitro blue tetrazolium).

Dissolved in 10 mM Tris-Hcl pH 8.5

(A) For basic lab based Western bl t test:

A small amount of tissue (approx. 0.1 grams) is taken from the suspect discs by needle biopsy, this is chopped up and removed into twice the volume (estimated) of extraction buffer and heated at 65°C for 10 minutes (the heating step may not be necessary). The extract is centrifuged to remove solid debris, and 40 µl is loaded onto SDS-gels and proteins separated by PAGE. The separated proteins are transferred and immobilised by electro-blotting onto nitro-cellulose (NC) or NC/nylon sandwich (NCN) membranes. The membrane is fixed in TCA solution for 1 minute and washed in blocking buffer for 2x 15 minutes. Then incubated with the conjugated antibody in blocking buffer (a 1 in 1000 dilution) for 15-30 minutes. After this time the membrane is washed 3x 5 minutes in 10 mM Tris pH 7.0, then incubated in the AP substrate solution for approx. 10 minutes. A positive reaction shows as a single dark band. This indicates a normal disc.

(B) For basic lab based dot blot test:

From needle biopsies from the suspect discs, a small amount of tissue (approx. 0.1 grams) is chopped up and removed into twice the volume (estimated) of extraction buffer and heated at 65°C for 10 minutes (the heating step may not be necessary). Solid debris is removed by centrifugation and approx. 10 µl of the extract is dotted onto a NC or NCN strip and placed in fixative for I minute, then washed in blocking buffer for 2X 15 minutes. The strip is then incubated with the conjugated antibody in blocking buffer (a 1 in 1000 dilution) for 15-30 minutes. After this time the strip is washed 3X 5 minutes in 10 mM Tris pH 7.0, then incubated in the AP substrate solution for approximately 10 minutes. A positive reaction shows as a single dark band. This indicates a normal disc.

(C) Enzyme linked immunosorbent assay (ELISA) test kit, not lab based:

The unconjugated monoclonal antibody to disc lysozyme C is immobilised onto a test strip (made of opaque polystyrene (same material as used for microtitre plates except opaque/white for visualisation) or NCN

sandwich or another support matrix which is able to bind antibodies. The test strip is pre-blocked once antibody has been immobilised. The tissue from the needle biopsies is chopped up and removed to 2X volumes of extraction buffer containing non-ionic detergent to keep protein native. The tissue is incubated in extraction buffer with occasional mixing for 15 minutes heated at 65°C (heating may not be needed). After this time 10-20 µl aliquots of extract is placed on the area of the test strip where the UC monoclonal antibody is immobilised, and left for 10-20 minutes for the lysozyme C to bind. The strip is then washed in excess 10 mM Tris pH 7.0 and 10-20 µl of the AP-conjugated monoclonal antibody placed on the same position of the strip. This is left for approx. 20 minutes and then washed in excess Tris pH 7.0 and then immersed in the substrate solution for 10 minutes. The presence of lysozyme C will be shown as a dark spot on the test strip, indicating a healthy disc.

D) Colorimetric assay:

Lysozyme is a β-N-acetylmuramidase which hydrolyses the β-1,4-glycosidic bond between N-acetylmuramic acid (MurNac) and N-acetylglucosamine (GlucNac). Colorimetric substracts for lysozyme C are synthesised. The first would comprise an indol based compound linked to N-acetylglucosamine by a β 1-4 linkage. This compound would be colourless. Once the lysozyme cleaves the linkage, the indol compound is released turning the reaction mixture coloured (probably blue). Also there is an alternative compound where the N-acetylmuramic acid is linked to an indol based compound to give an alternative colorimetric compound. This can be a qualitative assay or quantitative by measuring the absorbance of the released indol based compound at its peak absorbance when excited at its preferred excitation wavelength. This can then be converted to absolute values for lysozyme activity in the extract.

(2) ANNEXIN TYROSINE PHOSPHORYLATION DETECTION

Reagents different from (1):

a) 2 monocl nal antibodies ne to tyrosine phosphate which is conjugated to alkaline phosphatase (AP), and one to annexins which remains unconjugated (UC) and is used in the test strip only.

b) Extraction buffer: 10 mM Tris-Hcl pH 7.0
lmM EDTA
5mM sodium vanadate
1-3% non ionic detergent (e.g. TritonX100) for native
OR if denatured
Sodium dodecyl sulphate (SDS)

Exactly the same procedures (A, B, C) can be performed for the detection of annexin tyrosine phosphorylation. Except the test strip will have an unconjugated monoclonal antibody to annexin immobilised in the test area. This will bind the annexins for testing. A monoclonal antibody to tyrosine phosphate, conjugated to AP will be used as the detection antibody. The only other difference is that the extraction buffer will have 5 mM sodium vanadate to inhibit phosphatases.

(3) A COMBINED LYSOZYME C AND ANNEXIN TYROSINE PHOSPHORYLATION TEST KIT.

Lysozyme C and annexin tyrosine phosphorylation can be detected on one test strip. The test strip will have two areas outlined on it. 1) will have the lysozyme C monoclonal immobilised in it, 2) the other area will have annexin monoclonal immobilised in it. The tissue biopsy will be extracted with the buffer containing the sodium vanadate and 10-20 µl aliquots of extract is placed on each area of the test strip where the UC monoclonal antibodies are immobilised, and left for 10-20 minutes for the lysozyme C and annexins to bind to their respective areas. The protocol will be the same as outlined above, except the strips will be incubated with a mixture of AP conjugated monoclonal antibodies to lysozyme C and tyrosine phosphate. The final incubation in the AP substrate will give two dark dots for healthy tissue.

(4) PHOSPHATASE ACTIVITY IN TISSUE

Because the annexins are tyrosine dephosphorylated during disease progression/onset, there may be residual phosphatase activity still present in the tissue some time after the disease has started (assuming that in healthy tissue where there is no kinase activity, there is no phosphatase activity). It is assumed that this will eventually disappear with time (but on the other hand, it may be present throughout the disease). An additional test would be to test for phosphatase activity in the tissue. If phosphatase activity is present throughout the disease as a long term activity, then it gives us another disease marker. If however phosphatase activity disappears with time, then the absence of phosphatase activity and the absence of tyrosine phosphorylated annexins will indicate that the disease has been present for some time. The presence of phosphatase activity and the absence of tyrosine phosphorylated annexins will indicate a recent development of disease.

(A) Tissue biopsies will be extracted without sodium vanadate. 10-20 µl aliquots of extract will be placed on a blank area of a test strip (no immobilised antibodies in the defined area). This strip will bind all the proteins of the extract including the phosphatase. Aliquots of extract will be placed on the defined area of the test strip and left for 10-20 minutes. The immobilised phosphatase on the strip will be detected with the same AP substrate solution.

(B) A colorimetric assay which comprises placing aliquots of extract into a phosphatase substrate solution could also be used. The light absorbance would be measured at a certain wavelength. The substrate provides a detectable colour change and is a substrate for alkaline phosphatase.

(5) A COMMERCIAL TRIPLE TEST FOR LYSOZYME C ANNEXIN TYROSINE DEPHOSPHORYLATION AND PHOSPHATASE ACTIVITY

All three tests present on one strip. However, either the biopsies will have to be split between extraction with buffers with and without sodium

vanadate; or extract the tissue with ut sodium vanadate and hope that the annexins are not non-specifically dephosphorylated during extraction. Alternatively the lysozyme C and annexin could be on one strip and the phosphatase test on another. 10-20 µl aliquots of extract will be placed on two test strips. The first will be the strip described above in (3), the other will be a blank strip (no immobilised antibodies in the defined area). This second strip will bind all the proteins of the extract including the phosphatase. Aliquots of extract will be placed on the defined areas of each test strip and left for 10-20 minutes. The bound AP-antibodies on the one strip, and the immobilised phosphatase on the other strip will be detected with the same AP substrate solution. If phosphatase activity is present throughout the disease:- then this test strip will give 2 spots for healthy tissue (lysozyme C and tyrosine-phosphorylated annexins). Diseased tissue will give one spot for the phosphatase. If however, the phosphatase activity declines with time from disease onset, then there will be decreasing phosphatase activity with time, eventually tissue will have no phosphatase activity. In this case healthy tissue will have 2 spots (lysozyme C and annexin tyrosine phosphorylated with no phosphatase activity). Early diseased tissue will have one spot for phosphatase activity and late disease will have no spots at all.

(6) GENETIC TEST FOR LYSOZYME C DEFECT

Because lysozyme C is lost from diseased tissue there is the possibility that a genetic defect causes it to be deposited as amyloid in the tissue (known for other conditions). There is evidence that this condition does have a genetic component in many cases. There are known genetic changes in exon 2 of lysozyme C that causes the deposition of lysozyme C as amyloid. If this is indeed the process whereby lysozyme C is lost from tissues, then these or other mutations in the gene can be detected. Blood samples can be used to isolate a DNA sample for analysis by PCR with primers specific for the mutation.

The genes from a group of patients will be cloned by RTPCR using isolated RNA as template. The amplified gene will be sequenced to determine

what, if any, mutations are present and where they are. A set of primers will be synthesised with the genetic base change(s) identified from the patients incorporated into the 5' end of the primers (one base change per primer). So a number of mutations could be envisaged in different patients causing lysozyme loss (although it should be noted that there maybe only one mutation type which causes disease in all patients). In the test kit, a set primer for these mutations (a mixture of all primers) will be used in conjunction with a standard primer with no mutation in it (from a region of the gene with no mutation identified in all patients) to amplify gene fragments by PCR. If any of the identified mutations, are present then a DNA fragment of known size will be amplified, this will allow us to determine where the mutation is in the gene. If no mutation is present then no DNA fragment will be amplified because of the 5' mismatch.

Alternatively, primers can be synthesised, which have the non-mutant sequences in the 5' end for all mutation regions of the gene. Using this primer set as a mixture, with the standard non-mutant primer. DNA fragments can be amplified using PCR on DNA from blood. If there is no mutation then a ladder of bands of known sizes corresponding to amplifications from each of the primers in the mixture to the standard primer will be obtained. If on the other hand, a known mutation is present, one of the bands in the ladder will be missing. Not only will we know that there is a mutation but also where it is in the gene.

Sizes of DNA bands will be evaluated using standard agarose gel electrophoresis. The kit will comprise the primer sets. Buffers to extract DNA from blood and the reagents needed to carry out the PCR.

A similar process can be envisaged for any other process of lysozyme loss if there is a genetic cause, eg illegitimate activation of a ubiquitin pathway of lysozyme C degradation.

These tests may also be valid for other conditions such as degeneration

of joints, knees, hips, ankles etc., through a number of disease processes including arthritis. Samples will be the fluid from the joints in question.

(7) NUCLEAR MAGNETIC RESONANCE TECHNIQUES

It has been shown that lysozyme C is lost from the matrix of the disc nucleus during degenerative disease progression. The findings suggest that the lysozyme C may be lost through its deposition as insoluble amyloid and that this may be genetically determined/predisposed and degeneration triggered through some mechanical injury. Lysozyme C in the form of amyloid does not have enzymic activity therefore it is envisaged that this fact can be used to produce a non-invasive diagnostic for disc degeneration. One of the processes used in the diagnosis of degeneration of spinal discs is Nuclear Magnetic Resonance (NMR). A modification of the lysozyme substrate which comprises a substrate homologue with a beta 1-4 linkage is used to detect the diseased disc(s) by injecting the lysozyme C substrate analogue into the suspect discs or in tissue extracted therefrom, prior to NMR. If lysozyme C is present in its active form it will cleave the substrate thereby altering the magnetic resonance of the disc indicating a healthy disc. If however the lysozyme is deposited as amyloid or lost from the disc through some other mechanism - then the substrate will not be cleaved and there will be no observable change in the magnetic resonance of the disc indicating the presence of disease. In addition the degree of substrate cleavage may also be used to indicate the degree of disease progression, i.e. a low level of substrate cleavage would indicate some loss of lysozyme C and therefore possibly the start of disease. This technique may also be adapted such that the lysozyme substrate homologue could be injected systemically prior to NMR analysis. This way the presence of degenerative diseases in other joints could be diagnosed, i.e. osteoarthritis.

1) LYSOZYME C REPLACEMENT THERAPY

A) Working hypothesis:

Lysozyme C intradiscal replacement therapy restores the cellular/intracellular biochemical milieu that has been demonstrated to be altered in degenerate intervertebral discs derived from patients with low back pain and sciatica

B) Patient Selection criteria:

i) Back pain and/or sciatica unresponsive to conservative treatment:

Clinical symptoms:

- at least 6 months duration
- mild to moderate or appreciative functional disability

Diagnosis:

- supportive Magnetic resonance image scans showing disc degeneration and/or disc protrusion
- diagnostic test kit positive for Lysozyme C loss
- discography +ve for back pain +/- sciatic symptoms

ii) Sciatica unresponsive to conservative treatment:

Clinical symptoms:

- at least 6 months duration
- mild to moderate or appreciative functional disability

Diagnosis:

- supportive Magnetic resonance image scans showing disc protrusion
- diagnostic test kit positive for Lysozyme C loss
- discography +ve for sciatic symptoms

C) Therapy:

- using Image Intensifier (X-ray control)

- needle puncture of disc space under local anaesthetic
- determine whether disc pain +/- sciatic symptoms reproducible
- intradiscal injection of Lysozyme C performed

Lysozyme C therapy alone may not be sufficient. Further research may reveal that there are growth hormone(s) capable of inducing the intradiscal production of Lysozyme C. Both Lysozyme C protein and/or a known stimulatory hormone or protein may be required in this form of therapy.

2) PHOSPHATASE INHIBITOR THERAPY

A) Working hypothesis:

Specific or non-specific inhibition via the introduction of phosphatase inhibitor(s) prevents dephosphorylation of annexin I and II. This results in the restoration of control of PLA_2 and prevents the loss of cell growth and differentiation that have been demonstrated to be altered in degenerate intervertebral discs derived from patients with low back pain and sciatica

B) Patient Selection criteria:

i) Back pain and/or sciatica unresponsive to conservative treatment:

As per section 1) B) ii), but diagnostic test kit +ve for annexin dephosphorylation

ii) Sciatica unresponsive to conservative treatment:

As per section 1) B) ii), but diagnostic test kit +ve for annexin dephosphorylation

C) Therapy:

Intradiscal injection therapy:

- using Image Intensifier (X-ray control)
- needle puncture of disc space under local anaesthetic
- determine whether disc pain +/- sciatic symptoms reproducible
- intradiscal injection of phosphatase inhibitor performed

3) STEROID THERAPY

A) Working Hypothesis:

Various forms of corticosteroids are known to induce the production of both annexin I and II. This may be used in combination with the intradiscal introduction of either Lysozyme C alone or with Lysozyme C and phosphatase inhibitors.

4) GROWTH FACTOR THERAPY

A) Working Hypothesis:

Annexin I and II are the major substrates for tyrosine activity associated with growth factors (e.g. epidermal growth factor (EGF) and platelet derived growth factor (PDGF-R)), and hormones (e.g. insulin). The introduction of these various factors will restore the phosphorylation status of these annexin proteins.

5) COMBINATION THERAPY

The following combination therapy may be possible:

(1) = Lysozyme C
(2) = Phosphatase inhibitor
(3) = Steroid
(4) = Growth factor

i) (1) al ne
ii) (2) alone
iii) (1) + (2)
iv) (1) + (2) + (3)
v) (1) + (2) + (3) + (4)
vi) or any combination of (l), (2), (3), (4)

The Lysozyme C or phosphatase inhibitor therapy alone may not be sufficient and that treatment will probably require combination therapy with Lysozyme C and phosphatase inhibitor in conjunction with steroid and/or growth factor(s) therapy.

7) OTHER MEDICAL CONDITIONS

It appears that the aforementioned biochemical changes are not exclusive to patients with low back pain and sciatica, and may be seen in patients with degenerative joint diseases/arthritis, e.g. knee and hip osteoarthritis. Sample biopsies may be easily harvested from these joints, tested using the specific test kits, and treatment administered following the above guidelines.

Various modifications may be made without departing from the scope of the invention. For instance, the conjugated monoclonal antibodies may be conjugated to an enzyme other than alkaline phosphatase. Further, the administration of tyrosine phosphoylated proteins, in particular tyrosine phosphoylated annexin, could be used to treat the diseases addressed in this specification. Experimentation has revealed that there is a depletion of this product in the tissue and so replacement technique, such as injection into the tissue can be used to treat the diseases and conditions. The administration of tyrosine phosphoylated annexin may be carried out alone or in conjunction with one or more of the other substances referred to in this specification.

Whilst endeavouring in the forgoing specification to draw attention to the features of the invention believed to be of particular importance it should

be understood that the Applicant claims protection in respect of any patentable feature or combination of features hereinbefore referred to and/or shown in the drawings whether or not particular emphasis has been placed thereon.

Claims

1. A method of diagnosing low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint disease, or a predisposition thereto, said method comprising detecting the presence of lysozyme C in tissue extracted from intervertebral discs or fluid extracted from large joints.

2. A method as claimed in claim 1, in which the amount of lysozyme C is detected.

3. A method as claimed in any preceding claim, in which the method comprises western blotting, dot blotting, or an enzyme linked immunosorbent assay.

4. A method as claimed in any preceding claim, in which monoclonal antibodies to lysozyme C are used.

5. A method as claimed in claim 4, in which two antibodies are used, each of which recognises a different epitope of lysozyme C.

6. A method as claimed in claim 5, in which both antibodies recognise denatured and native protein.

7. A method as claimed in claim 5 or claim 6, in which one of said antibodies is conjugated to an enzyme such as alkaline phosphatase with the other antibody remaining unconjugated.

8. A method as claimed in any preceding claim, in which a colour change occurs when lysozyme C is present.

9. A method as claimed in claim 8, in which a substrate for the enzyme provides the colour change in the presence of lysozyme C.

10. A method as claimed in any of claims 7 to 9, in which only the conjugated monoclonal antibody is used in western blotting and dot blotting.

11. A method as claimed in any of claims 7 to 10, in which the conjugated antibody and the unconjugated antibody are utilised in the enzyme linked immunosorbent assay.

12. A method as claimed in any preceding claim, in which the method comprises a colorimetric assay.

13. A method as claimed in claim 12, in which colorimetric substrates for lysozyme C are synthesised for use in the assay.

14. A method as claimed in claim 13, in which the substrate is an indol based compound linked to N-acetylglucosamine by a β 1-4 linkage.

15. A method as claimed in claim 13 or claim 14, in which the substrate is clear and the mixture of substrate and lysozyme C becomes coloured when lysozyme C cleaves the linkage.

16. A method as claimed in claim 13, in which the substrate is a compound where N-acetylmuramic acid is linked to an indol based compound.

17. A method as claimed in any of claims 14 to 16, in which the absorbance of the released indol based compound is measured at its peak absorbance when excited at its excitation wavelength.

18. Apparatus for use in the diagnosis of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said apparatus comprising a support on which an unconjugated monoclonal antibody to lysozyme C is immobilised whereby an indication is given when the support comes into contact with lysozyme C.

19. Apparatus as claimed in claim 18, in which the support comprises a test strip or a nitro-cellulose/nylon sandwich membrane.

20. Apparatus as claimed in claim 19, in which the test strip comprises opaque polystyrene or another support matrix which is able to bind antibodies.

21. Apparatus as claimed in any of claims 18 to 20, in which the support is pre-blocked with blocking agents when the unconjugated antibody has been immobilised thereon.

22. Apparatus as claimed in any of claims 18 to 21, in which the apparatus is utilised in an enzyme linked immunosorbent assay using an enzyme conjugated monoclonal antibody to lysozyme C.

23. Apparatus as claimed in claim 22, in which the enzyme is alkaline phosphatase, horse radish peroxidase or biotin.

24. Apparatus as claimed in claim 22 or claim 23, in which the substrate for the enzyme provides a colour change as an indication of the presence of lysozyme C.

25. A method of treatment of low back pain and sciatica, comprising the administration, to a body in need of such treatment, of an effective dose of lysozyme C.

26. A method as claimed in claim 25, in which the lysozyme C is administered by injection into a vertebral disc or joint.

27. A method as claimed in claim 25 or claim 26, in which the lysozyme C is administered in combination with a growth factor, tyrosine phosphorylated protein and/or a phosphatase inhibitor.

28. A method as claimed in any of claims 25 to 27, in which the method is

used to treat joint disease.

29. A method of diagnosing low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said method comprising detecting the presence of tyrosine phosphorylation in tissue extracted from intervertebral discs or fluid extracted from large joints.

30. A method as claimed in claim 29, in which the amount of tyrosine phosphorylation is detected.

31. A method as claimed in claim 29 or claim 30, in which the method involves detecting the presence of annexin tyrosine phosphorylation.

32. A method as claimed in any of claims 29 to 31, in which the method comprises western blotting, dot blotting, or an enzyme linked immunosorbent assay.

33. A method as claimed in claim 32, in which two monoclonal antibodies are used in the tests.

34. A method as claimed in claim 33, in which the first antibody recognises tyrosine phosphate and is conjugated to an enzyme.

35. A method as claimed in claim 33 or claim 34, in which the second antibody recognises annexins and is unconjugated.

36. A method as claimed in any of claims 33 to 35, in which the first antibody is conjugated to alkaline phosphatase, horse radish peroxidase or biotin.

37. A method as claimed in any of claims 31 to 36, in which a colour change occurs when a tyrosine phosphorylated annexin is present.

38. A method as claimed in any of claims 34 to 37, in which a substrate for the enzyme provides the colour change in the presence of a tyrosine phosphorylated annexin.

39. A method as claimed in any of claims 29 to 38, in which a phosphatase inhibitor is utilised when extracting the tissue.

40. A method as claimed in claim 39, in which the inhibitor is sodium vanadate.

41. A method as claimed in any of claims 34 to 40, in which only the conjugated monoclonal antibody is used in western blotting and dot blotting.

42. A method as claimed in any of claims 34 to 41, in which the conjugated antibody and the unconjugated antibody are utilised in the enzyme linked immunosorbent assay.

43. Apparatus for use in the diagnosis of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said apparatus comprises a support on which an unconjugated monoclonal antibody to annexin is immobilised whereby an indication is given when the support comes into contact with tyrosine phosphorylated annexin.

44. Apparatus as claimed in claim 43, in which the support comprises a test strip or a nitro-cellulose/nylon sandwich membrane.

45. Apparatus as claimed in claim 44, in which the test strip comprises opaque polystyrene or another support matrix which is able to bind antibodies.

46. Apparatus as claimed in claim 43 or claim 44, in which the support is pre-blocked with blocking agents when the unconjugated antibody has been immobilised thereon.

47. Apparatus as claimed in any of claims 43 to 46, in which the apparatus is utilised in an enzyme linked immunosorbent assay using an enzyme conjugated monoclonal antibody to tyrosine phosphate.

48. Apparatus as claimed in claim 47, in which the enzyme is alkaline phosphatase, horse radish peroxidase or biotin.

49. Apparatus as claimed in claim 47 or claim 48, in which the substrate for the enzyme provides a colour change in the presence of tyrosine phosphorylated annexin.

50. Apparatus for use in the diagnosis of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said apparatus comprising a support on which an unconjugated monoclonal antibody to annexin and an unconjugated antibody to lysozyme C are immobilised whereby an indication is given when the support comes into contact with tyrosine phosphorylated annexin and lysozyme C.

51. Apparatus as claimed in claim 50, in which the two antibodies are immobilised on separate areas of the support.

52. Apparatus as claimed in claim 50 or claim 51, in which the support comprises a test strip or a nitro-cellulose/ nylon sandwich membrane.

53. Apparatus as claimed in claim 52, in which the test strip comprises opaque polystyrene or another support matrix which is able to bind antibodies.

54. Apparatus as claimed in any of claims 50 to 53, in which the support is pre-blocked with blocking agents when the unconjugated antibodies have been immobilised thereon.

55. Apparatus as claimed in any of claims 50 to 54, in which the apparatus is

utilised in an enzyme linked immunos rbent assay using enzyme conjugated monoclonal antibodies to lysozyme C and tyrosine phosphate.

56. Apparatus as claimed in claim 55, in which the enzyme is alkaline phosphatase, horse radish peroxidase or biotin.

57. Apparatus as claimed in claim 55 or claim 56, in which the substrate for the enzyme provides a colour change in the presence of tyrosine phosphorylated annexin and lysozyme C.

58. A method of diagnosing low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said method comprising detecting the presence of phosphatase in tissue extracted from intervertebral discs or fluid extracted from large joints.

59. A method as claimed in claim 58, in which the amount of phosphatase is detected.

60. A method as claimed in claim 58 or claim 59, in which the method comprises placing aliquots of extract on a blank area of a test strip or a nitro-cellulose/nylon sandwich membrane.

61. A method as claimed in claim 60, in which the test strip comprises opaque polystyrene or another support matrix which is able to bind antibodies.

62. A method as claimed in claim 60 or claim 61, in which the extract is left on the test strip or membrane for a predetermined period of time and preferably for 10-20 minutes.

63. A method as claimed in any of claims 60 to 62, in which a colour change occurs when there is immobilised phosphatase on the test strip or membrane.

64. A method as claimed in claim 63, in which a substrate for alkaline phosphatase provides the colour change in the presence f immobilised phosphatase.

65. A method as claimed in any of claims 58 to 64, in which the method comprises a colorimetric assay.

66. A method as claimed in claim 65, in which the assay comprises placing aliquots of extract into a phosphatase substrate solution and measuring the light absorbance at a certain wavelength.

67. A method as claimed in claim 66, in which the substrate is a substrate for alkaline phosphatase which provides a colour change in the presence of phosphatase.

68. A method of treatment of low back pain and sciatica, comprising the administration, to a body in need of such treatment, of an effective dose of phosphatase inhibitor.

69. A method as claimed in claim 68, in which the phosphatase inhibitor is administered by injection into a vertebral disc or joint.

70. A method as claimed in claim 68 or claim 69, in which the inhibitor is administered in combination with lysozyme C.

71. A method as claimed in any of claims 68 to 70, in which the method is also used to treat joint disease.

72. A method of treatment of low back paid and sciatica, comprising the administration, to a body in need of such treatment, of an effective dose of tyrosine phosphorylated annexin.

73. A method as claimed in claim 72, in which the method may be used to

treat joint disease.

74. A method of diagnosing low back paid and sciatica, or a predisposition thereto, the method comprising analysing the presence of tyrosine phosphorylation or phosphatase and of lysozyme C in tissue extracted from the invertebral discs or fluid extracted from large joints.

75. The use of lysozyme C and a phosphatase inhibitor in the manufacture of a medicament for the treatment of low back pain and sciatica.

76. The use of lysozyme C as claimed in claim 75, in which it may be used to treat joint disease.

77. A method of diagnosing low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said method comprising detecting the presence of mutations in the lysozyme C gene in blood samples taken from the body.

78. A method as claimed in claim 77, in which the method comprises using a set primer for these mutations in conjunction with a standard primer with no mutation to amplify the gene fragments by the Polymerase Chain Reaction (PCR) if the mutation is present.

79. A method as claimed in claim 77, in which the method comprises synthesising primers with the non-mutant sequences in the 5' end for all mutation regions of the lysozyme C gene.

80. A method as claimed in claim 79, in which the set of primers is used as a mixture thereby enabling DNA fragments to be amplified using PCR on the blood DNA to produce a DNA profile.

81. A method as claimed in any of claims 77 to 80, in which the absence of certain bands from the profile is indicative of disease.

82. Apparatus for use in the diagnosis of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said apparatus comprising a set of primers with non mutant sequences in the 5' end for all mutation regions of the lysozyme C gene for use in PCR amplification of DNA fragments.

83. Apparatus as claimed in claim 82, in which the apparatus is utilised for detecting the presence of mutations in the lysozyme C gene.

84. Use of lysozyme C as a therapeutic agent in the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases.

85. A method as claimed in claim 84, in which the lysozyme C is injected into the disc or joint.

86. A method as claimed in claim 85, in which the lysozyme C is injected in combination with a growth factor.

87. The use of lysozyme C in the manufacture of medicament for the treatment of back pain and sciatica.

88. Use of a phosphatase inhibitor as a therapeutic agent in the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases.

89. The use of a phosphatase inhibitor in the manufacture of a medicament for the treatment of back pain and sciatica.

90. Use of corticosteroids as a therapeutic agent in the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases.

91. The use of corticosteroids for use in the manufacture of a medicament for the treatment of back pain and sciatica.

92. The use of growth factors as therapeutic agents in the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases.

93. Use according to claim 92, in which the growth factors are epidermal growth factor, platelet derived growth factor or hormones such as insulin, or any agonist which will restore the tyrosine phosphorylation of annexins.

94. The use of tyrosine phosphorylation annexins in the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint disease.

95. Use according to claim 94, in which the growth factors are injected in combination with any or all of lysozyme C, phosphatase inhibitor and corticosteroids.

96. The use of tyrosine phosphoylated annexins in the manufacture of a medicament for the treatment of low back pain and sciatica.

97. The use of growth factors in the manufacture of a medicament for the treatment of back pain and sciatica.

98. The use of any one or combination of lysozyme C, phosphatase inhibitor, corticosteroids, growth factors and tyrosine phosphoylated annexins in the manufacture of a medicament for the treatment of low back pain, sciatica and joint disease.

99. A pharmaceutical composition comprising any one of a combination of lys zyme C, phosphatase inhibitor, corticosteroid, growth factor and tyrosine phosphorylated annexin in association with a pharmaceutically acceptable

diluent r carrier.

100. A composition as claimed in claim 99, in which the composition is used for the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint disease, or a predisposition thereto.

101. A method of diagnosing low back pain and sciatica associated with degenerative disc disease and degenerative joint disease, or a predisposition thereto, said method comprising introducing a lysozyme C substrate analogue in tissue of intervertebral discs or large joints and analysing the magnetic resonance of the substrate with nuclear magnetic resonance techniques.

102. A method as claimed in claim 101, in which the analogue is introduced by injection.

103. A method as claimed in claim 101 or 102, in which the analogue comprises a substrate homologue with a beta 1-4 linkage.

104. A method as claimed in any of claims 101 to 103 in which the method involves analysis of the degree of change in the magnetic resonance properties of the homologue, which may provide for determination of the degree of disease progression.

105. The use of lysozyme C substrate analogue in the analysis of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases.

106. The use of lysozyme C substrate analogue as claimed in claim 105, in which the magnetic resonance characteristics of analogue introduced into tissue of intervertebral discs and joints are analysed using nuclear magnetic res nance techniques.

107. The use f lysozyme C substrate analogue as claimed in claim 105 or claim 106, in which the analogue is injected into tissue to be analysed.

108. The use of lysozyme C substrate analogue as claimed in any of claims 105 to 107, in which the analogue is introduced systemically to a body to be analysed.

109. Any novel subject matter or combination including novel subject matter disclosed herein, whether or not within the scope of or relating to the same invention as any of the preceding claims.

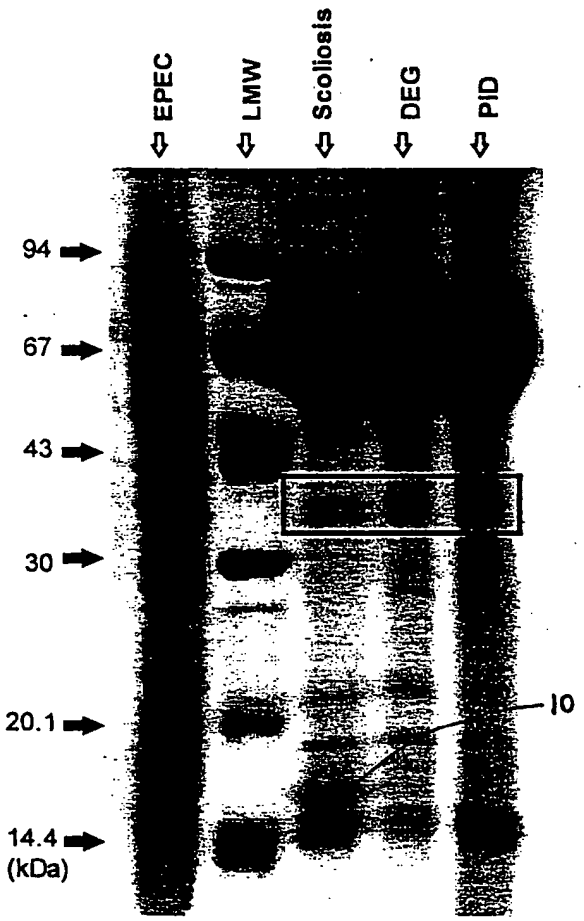
EPEC
LMW
Scoliosis
DEG
PID
94
67
43
30
20.1
14.4
(kDa)
10
(a)

FIG. 1

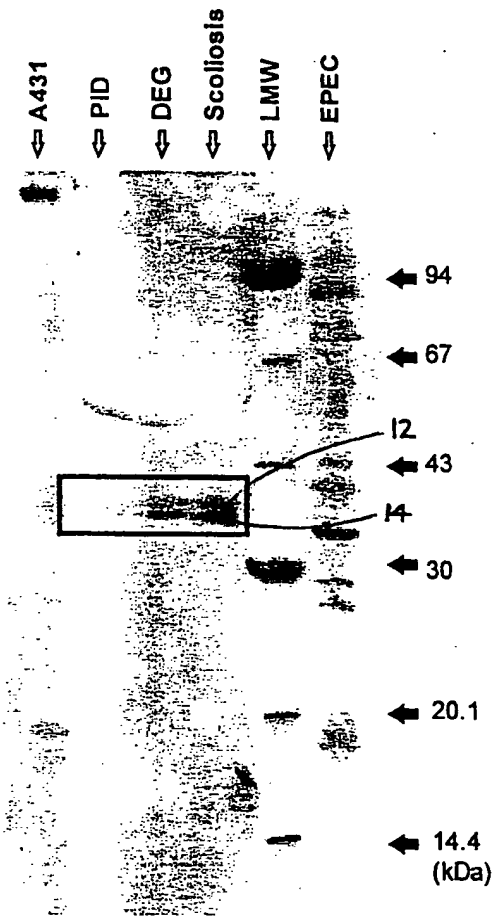
A431
PID
DEG
Scoliosis
LMW
EPEC
94
67
43
30
20.1
14.4
(kDa)
12
14
(b)

FIG. 2